



**IN THE UNITED STATES PATENTS AND  
TRADEMARKS OFFICE**

**APPLICANT: LUTZ HEUER ET AL**  
**SERIAL NO.: 09/901,979**  
**FILED: JULY 10, 2001**  
**FOR: MICROBICIDAL COMPOSITIONS**

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**DECLARATION**

I, Martin Kugler, resident at Am Kloster 47, D-42799 Leichlingen, Germany declare:

that I am a biologist having studied at the University of Tübingen;

that I received the degree of doctor rer. nat. at the University of Tübingen in the year of 1986;

that since 1987 I am an employee of Bayer Aktiengesellschaft, Leverkusen, Germany, where I am working in the department of research of technical materials protecting agents located at Krefeld, Germany;

that I am a senior research microbiologist having 16 years experiences in the field of testing and evaluation of chemical compounds for their antimicrobial action;

that I am one of the inventors of the above-identified application;

that the following tests have been carried out under my supervision and direction:

## Test 1

### **Synergism of Cyproconazole / Propiconazol**

Pieces of mycelium were punched out of a colony of the wood-destroying fungus **Lentinus tigrinus** and incubated on a nutrient agar containing malt extract/peptone at 26°C. The hyphal growth with and without the addition of the active compound was compared. The minimum inhibitory concentration (MIC) was recorded as the concentration of active compound which completely suppresses radial hyphal growth.

According to the method described by Kull et al. (F.C. Kull, P.C. Eismann, H.D. Sylvestrowicz, R. L. Mayer, Applied Microbiology 9, 538 to 541, 1961) the synergism was then determined. The following equation for the determination of the synergistic index X applies:

$$\frac{Q_A}{Q_a} + \frac{Q_B}{Q_b} = X$$

X = 1 = additivity

X > 1 = antagonism

X < 1 = synergism

$Q_a$  = the MIC of substance A

$Q_b$  = the MIC of substance B

$Q_A$  = the concentration of substance A in the concentration of A/B which suppresses microbial growth

$Q_B$  = the concentration of substance B in the concentration of A/B which suppresses microbial growth

## Results:

		MIC
A	cyproconazole	0.5 ppm
B	propiconazole	1 ppm
A/B	cyproconazole/propiconazole	0.5 ppm
(1/2)		

By using the above values for cyproconazole/propiconazole, mixing ratio of 1:2, the synergistic index X was determined:

$$X = \frac{0.17}{0.5} + \frac{0.33}{1.0} = 0.67$$

Thus, when cyproconazole and propiconazole are mixed in a ratio of 1:2 a high degree of synergism occurs.

## Test 2

### **Synergism of Cyproconazole / Tebuconazole**

Pieces of mycelium were punched out of a colony of the wood-destroying fungus **Lentinus tigrinus** and incubated on a nutrient agar containing malt extract/peptone at 26°C. The hyphal growth with and without the addition of the active compound was compared. The minimum inhibitory concentration (MIC) was recorded as the concentration of active compound which completely suppresses radial hyphal growth.

The synergistic index X was determined according to the method described in Test 1.

Results:

		MIC
A	cyproconazole	0.5 ppm
B	tebuconazole	0.3 ppm
A/B (2/1)	cyproconazole/tebuconazole	0.3 ppm

$$x = 0.73$$

Thus, when cyproconazole and tebuconazole are mixed in a ratio of 2:1 a high degree of synergism occurs.

Test 3

**Synergism of Cyproconazole / copper chloride**

Pieces of mycelium were punched out of a colony of the wood-destroying fungi **Coriolus versicolor** and incubated on a nutrient agar containing malt extract/peptone at 26°C. The hyphal growth with and without the addition of the active compound was compared. The minimum inhibitory concentration (MIC) was recorded as the concentration of active compound which completely suppresses radial hyphal growth.

The synergistic index X was determined according to the method described in Test 1.

Results:

	MIC
A cyproconazole	0.5 ppm
B copper chloride	>100 ppm
A/B cyproconazole/copper chloride (1/25)	7 ppm

$$x = 0.63$$

The results show that a high degree of synergism occurs when using cyproconazole and copper chloride in a mixing ratio of 1:25 against the wood-destroying fungus *Coriolus*.

Test 4

**Synergism of Cyproconazole / basic copper(II) carbonate**

Pieces of mycelium were punched out of a colony of the wood-destroying fungi ***Coriolus versicolor*** and incubated on a nutrient agar containing malt extract/peptone at 26°C. The hyphal growth with and without the addition of the active compound was compared. The minimum inhibitory concentration (MIC) was recorded as the concentration of active compound which completely suppresses radial hyphal growth.

The synergistic index X was determined according to the method described in Test 1.

Results:

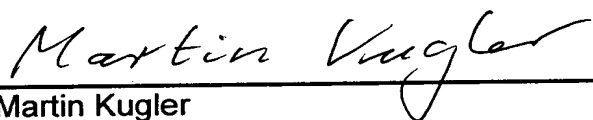
A	cyproconazole	MIC 0.5 ppm
B	basic copper(II) carbonate	>100
A/B	cyproconazole/basic copper(II) carbonate (1/25)	7

$$x = 0.63$$

The results show that a high degree of synergism occurs when using cyproconazole and basic copper(II) carbonate in a mixing ratio of 1:25 against the wood-destroying fungus *Coriolus*.

The undersigned declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Leverkusen, Germany, this      day of      2002 - 09 - 24

  
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Martin Kugler